The Effect of Leukocytic Endogenous Mediator (LEM) on Serum Copper and Ceruloplasmin Concentrations in the Rat[1] (36926)

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Recent studies have demonstrated that stimulated leukocytes release an endogenous mediator or mediators, which upon administration to normal rats induces the following alterations in host metabolism: (a) a decrease in both serum zinc and iron concentrations (1–3); (b) an increase of Zn and Fe within the liver (4); (c) flux of amino acids to the liver (5); (d) an increase in serum α_1- and α_2-acute phase globulins (6); and (e) an accelerated release of neutrophils from bone marrow (7).

Other inflammation-induced changes in host metabolism include increases in serum copper (8–11), ceruloplasmin (8, 9, 11), alterations in serum proteins and glycoprotein patterns (12–15) and the synthesis of specific proteins such as seromucoid, haptoglobulin, fibrinogen and protein-bound hexoses (15). The present investigation was undertaken to determine whether LEM could also cause a change in several of these parameters, namely, the increase of serum copper and ceruloplasmin and synthesis of serum protein.

Materials and Methods. Healthy adult male New Zealand rabbits, weighing 2.5–3.0 kg, and healthy male Dunning-Fisher rats weighing 150–175 g, were used in this study. The LEM preparation was obtained by infusing rabbits ip with a pyrogen-free normal saline solution containing 0.2% shellfish glycogen (Mann Research Laboratories, New York, NY). Peritoneal exudates were collected aseptically 12 hr after infusion, and polymorphonuclear (PMN) leukocytes were obtained by centrifugation. The harvested cells were resuspended in pyrogen-free normal saline and incubated at 37° for 2 hr (3–4). The supernatant obtained after centrifugation represented the crude LEM preparation. The protein concentration of the preparation was determined by the method of Lowry *et al.* (16).

To test the effect of LEM on serum Cu, ceruloplasmin and serum protein synthesis, 1 ml of the crude LEM preparation, containing 1 mg protein/ml, was administered ip to a group of rats. An equal portion of the LEM was heat inactivated (90° for 30 min) and administered ip to another group of rats. This was done as a control to insure that observed changes were not due to any contaminating heat-stable endotoxins. A third group of rats were inoculated with an equal volume of sterile pyrogen-free normal saline and served as an added set of controls. In order to study amino acid incorporation into total serum proteins, all rats were given ip 10 μCi of L-4,5-^{3}H-leucine (55.5 μCi/mmole, New England Nuclear, Boston, MA)/100 g body wt 2 hr before the rats were to be killed.

At the times designated in Fig. 1, rats from each group were anesthetized with 2-bromo-2-chloro-1,1,1-trifluorethane (halothane), bled from the axillary fold pouch by severing the brachial artery, and then killed by cervical dislocation. The individual serum samples were subsequently divided into 3 aliquots for the determination of serum Cu and ceruloplasmin concentrations and for measurement of the incorporation of ^{3}H-leucine into serum proteins.

Serum Cu concentrations were determined by an atomic absorption spectrophotometric

[1] In conducting the research described in this report, the investigators adhered to the "Guide for Laboratory Animal Facilites and Care," as promulgated by the Committee on the Guide for Laboratory Animal Facilities and Care of the Institute of Laboratory Animal Resources, National Academy of Sciences-National Research Council. The facilities are fully accredited by the American Association of Accreditation of Laboratory Animal Care.

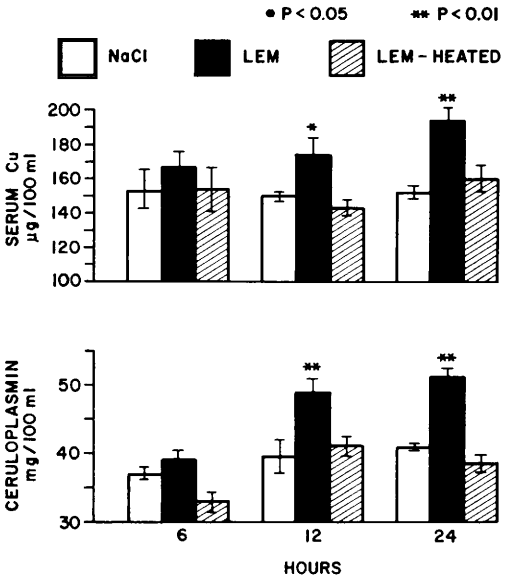

FIG. 1. The effect of LEM on serum Cu and ceruloplasmin concentrations in the rat at 6, 12 and 24 hr postadministration. Values are expressed as the mean ± SE.

technique described by Pekarek *et al.* (10), and ceruloplasmin by the method of Ravin (17). Serum samples for the determination of ^{3}H-leucine incorporation into total serum proteins were treated and prepared for counting by the method described by Powanda, Wannemacher and Cockerell (14), and were counted in a 3-channel Nuclear Chicago scintillation counter with external standardization.

Results and Discussion. As shown in Fig. 1, a single injection of LEM at zero time elicited some increase in both serum Cu and ceruloplasmin by 6 hr post administration. By 12 hr significant increases in both parameters were observed in the test group when compared to either the heat-inactivated LEM or saline control groups. The increase in circulating ceruloplasmin corresponds to a significant, but transient, increase in the incorporation of ^{3}H-leucine into total serum proteins observed at 12 hr postadministration (Table I).

Although LEM induces an increase in serum Cu concentrations, this alteration in Cu metabolism does not become readily apparent until 12 hr postadministration. By contrast, LEM induced alterations in serum Zn and Fe concentrations in the rat have been shown to occur within 2–3 hr and become maximal by 6 hr after the administration of LEM (3). A similar chronology of events has been observed in prospective clinical studies in volunteers receiving either sandfly fever virus (18) or live attenuated Venezuelan equine encephalomyelitis virus vaccine (10). The increase in serum Cu in these studies were slightly delayed when compared to the lowering serum Zn and Fe concentrations or the depression in serum amino acids, especially those amino acid which have been shown to be necessary for protein synthesis. The delay in the release of Cu into the serum is most likely due to the time necessary to produce increased amounts of ceruloplasmin. Ceruloplasmin synthesis has been shown to be necessary for the removal of Cu from its storage pool in the liver (19), and Kleinbaum (9) has reported that infection-induced increases in serum Cu concentrations were in the indirect reacting Cu fraction (ceruloplasmin-bound Cu) and were related to the stimulation of apoceruloplasmin synthesis.

These preliminary data on serum protein metabolism presented in Table I suggest that LEM transiently stimulates the hepatic synthesis and release of some serum proteins. Although the rate of serum protein synthesis returned to control levels by 24 hr, increased concentrations of ceruloplasmin, an α_2-glycoglobulin with a half-life of 5–7 days (20), continued to be present in the circulation.

TABLE I. The Effect of LEM on the Incorporation of ^{3}H-Leucine into Serum Proteins.

Treatment	Postadministration (hr)	^{3}H-Leucine (dpm/µg protein)[a]
LEM	6	38.7 ± 4.3
LEM heated control	6	41.8 ± 2.5
LEM	12	53.9 ± 4.7[b]
LEM heated control	12	36.1 ± 5.1
LEM	24	38.1 ± 4.8
LEM heated control	24	40.1 ± 3.3

[a] Means ± SE.

[b] Value significantly different from heat inactivated control, $p < .01$.

The increase and persistence of ceruloplasmin concentrations found in this study closely correspond in timing to the increase and persistence of α_2-acute phase globulin concentrations in rat serum, which appear within 10 hr after the administration of LEM (6). The α_2-acute phase globulin is either absent or at extremely low values in normal rat serum, but, like ceruloplasmin, it can be stimulated by a variety of inflammatory stresses such as infection, endotoxemia, tumors, turpentine abscess and irradiation (15). In addition, the serum seromucoid fraction, which has been shown to be increased during inflammation (15, 21), also has been observed to be increased following the administration of LEM to rats (personal communication, M. C. P.).

This endogenous mediator has been shown to be released by PMN leukocytes (1–7). However, this does not preclude the possibility that other cell types may produce such a mediator. Since LEM shares certain chemical and physical properties with endogenous pyrogen (3), which has been shown to be released in different degrees by a number of different cell types (22), work has been extended to study the release of such a mediator by other cell types, as well as its specificity in producing the metabolic responses as described for LEM. Preliminary studies have shown that only properly stimulated monocytes and macrophages liberate a similar mediating factor (personal communication, R. S. P.).

Recently, Weimer, Roberts and Comb (23), have discussed the absence of experimental data to demonstrate a biochemical mediator, which influences plasma protein synthesis *in vivo* during the inflammatory process. The data presented in this study, however, suggest that LEM may be such a mediator. Although the overall importance of the endogenous mediator or its effects are not fully understood, it appears that this humoral factor may be a key intermediate in stimulating a number of nonfebrile host responses during infection and inflammation.

Summary. Leukocytic endogenous mediator (LEM) was shown to produce significant increases in serum Cu and ceruloplasmin concentrations in the rat within 12 hr after its administration. This increase coincided with an increase in the incorporation of ^{3}H-leucine into total serum proteins which also was shown to be induced by LEM.

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